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## Effects of dietary protein content on IGF-I, testosterone, and body composition during 8 days of severe energy deficit and arduous physical activity

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**Alemany JA, Nindl BC, Kellogg MD, Tharion WJ, Young AJ, Montain SJ.** Effects of dietary protein content on IGF-I, testosterone, and body composition during 8 days of severe energy deficit and arduous physical activity. *J Appl Physiol* 105: 58–64, 2008. First published May 1, 2008; doi:10.1152/jappphysiol.00005.2008.—Energy restriction coupled with high energy expenditure from arduous work is associated with an altered insulin-like growth factor-I (IGF-I) system and androgens that are coincident with losses of fat-free mass. The aim of this study was to determine the effects of two levels of dietary protein content and its effects on IGF-I, androgens, and losses of fat-free mass accompanying energy deficit. We hypothesized that higher dietary protein content would attenuate the decline of anabolic hormones and, thus, prevent losses of fat-free mass. Thirty-four men [24 (SD 0.3) yr, 180.1 (SD 1.1) cm, and 83.0 (SD 1.4) kg] participated in an 8-day military exercise characterized by high energy expenditure (16.5 MJ/day), low energy intake (6.5 MJ/day), and sleep deprivation (4 h/24 h) and were randomly divided into two dietary groups: 0.9 and 0.5 g/kg dietary protein intake. IGF-I system analytes, androgens, and body composition were assessed before and on days 4 and 8 of the intervention. Total, free, and nontertiary IGF-I and testosterone declined 50%, 64%, 55%, and 45%, respectively, with similar reductions in both groups. There was, however, a diet  $\times$  time interaction on day 8 for total IGF-I and sex hormone-binding globulin. Decreases in body mass (3.2 kg), fat-free mass (1.2 kg), fat mass (2.0 kg), and percent body fat (1.5%) were similar in both groups ( $P = 0.01$ ). Dietary protein content of 0.5 and 0.9 g/kg minimally attenuated the decline of IGF-I, the androgenic system, and fat-free mass during 8 days of negative energy balance associated with high energy expenditure and low energy intake.

soldier; caloric deprivation; sleep restriction; underfeeding; body composition

PHYSICALLY DEMANDING OCCUPATIONS often require periods of sustained work without adequate caloric and dietary protein intake. The resultant negative energy and nitrogen balance can lead to physical (12, 19, 20) and cognitive (7) performance decrements, as well as loss of muscle mass (5, 8, 18, 19, 26). For example, during chronic energy restriction (62 days), body mass decreased by 12%, fat-free mass decreased by 6% and fat mass by 50% and these changes were coincident with a 20% decline in strength and power (17). Significant losses of body mass, fat-free mass, and lower body physical performance have occurred in as little as 3 days (20). Greater resolution of the temporal kinetics of catabolism during such scenarios is re-

quired, inasmuch as it may allow for the appropriate identification of countermeasures. Although individuals engaged in strenuous physical activity (resistance and/or endurance training) are advised to consume additional dietary protein to sustain protein synthesis and nitrogen balance (3), it is unclear whether normalization of protein intake from an inadequate protein level to one considered adequate would attenuate the loss of fat-free mass accompanying an energy deficit (31).

A provocative hypothesis is that the decline of circulating anabolic hormones during an acute energy deficit can be attenuated by an increase in the protein content of the diet. Friedl et al. (5) and Guezennec et al. (8) demonstrated that increased caloric intake during military field operations helped preserve circulating concentrations of insulin-like growth factor-I (IGF-I) and testosterone, as well as fat-free mass. However, when total energy intake was increased by carbohydrate alone, anabolic hormone reductions were only modestly attenuated (10, 28). These observations suggest that dietary protein supplementation during periods of energy restriction may have positive effects on growth factors and anabolic hormones and may possibly mitigate losses of fat-free mass and physical performance (29, 31). An attenuation of the decline of circulating anabolic growth factors may serve to protect the muscle from degradation through the preservation of cellular signaling pathways associated with protein synthesis (i.e., the Akt-mammalian target of rapamycin signaling system). Increasing the dietary protein content has been considered a possible countermeasure, in light of the Military Recommended Dietary Intake and the fact that a significant proportion of meals-ready-to-eat (MREs) actually provide <0.9 g/day of dietary protein. For physically active populations that have high energy expenditures, higher-protein diets (1.2–1.8 g/kg body wt) are generally recommended (3, 31). For example, Picosky et al. (31) used diets that contained 0.9 and 1.8 g/kg of dietary protein during a 7-day exercise-induced energy deficit (–4.2 MJ/day) and noted that the higher-protein diet mitigated a decrease in nitrogen balance (31).

IGF-I is key regulator of metabolism during altered energy states and circulates in three distinct forms: 1) a 150-kDa ternary complex of IGF-I + IGF-binding protein (IGFBP)-3 and the acid-labile subunit (ALS), 2) a 20- to 35-kDa binary complex of IGF-I (IGF-I + IGFBP-1 or -2), and 3) and a 7.5-kDa free form of IGF-I. The ternary complex is a reservoir

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for IGF-I and is confined to the circulation, whereas binary and free IGF-I can pass through the vasculature toward target tissues. During periods of negative energy intake, these lower-molecular-weight binding proteins (IGFBP-1 and -2) typically increase (13, 18, 36), whereas the singular components of the ternary system (e.g., total IGF, IGFBP-3, and ALS) decrease (7, 18, 32). The physiological significance of these response patterns is that IGF-I bioavailability is regulated by the relative proportions of IGF-I in various molecular complexes (18, 22, 30, 35). From these observations, it may be inferred that redistribution occurs, shifting the relative proportions of IGF-I among its complexes. Using high-performance liquid chromatography, Thissen et al. (35) demonstrated decreases in IGF-I ternary complexes, with a concomitant increase in binary forms of IGF-I after energy and/or protein restriction, in a rat model. However, examination of ternary vs. binary IGF-I complexes after a 4-day military operation by a novel magnetic separation technique demonstrated no change in the relative distribution of IGF-I in ternary and binary complexes (18). It is not known whether a greater degree of energy strain (i.e., duration and/or magnitude of energy deficit) or macronutrient manipulation can alter the relative distribution of IGF-I among its molecular complexes.

The purpose of the present study was to determine whether differences in dietary protein would attenuate reductions of the IGF-I system, testicular and adrenal androgens, and fat-free mass losses accompanying several days of arduous work and energy deficit. The dietary protein intake of individuals participating in an 8-day field-training exercise was manipulated in two groups of volunteers consuming an isocaloric diet that contained two levels of protein: 38 and 82 g/day. The rationale for these two protein levels was that the currently available military MRE is generally low in protein. We purposefully sought to determine the effects of a nominal difference in protein content, i.e., 0.5 vs. 0.9 g/kg, on anabolic/metabolic hormones and body composition. For example, seven MRE menus contain <34 g of protein (mean 5.3 MJ and 32 g of protein), and seven MRE menus contain >49 g of protein (mean 5.3 MJ and 53 g of protein). It was hypothesized that 0.9 g/kg of protein would attenuate the reductions in IGF-I and testosterone concentrations accompanying negative energy balance compared with a lower-protein (i.e., 0.5 g/kg) diet and that these responses would result in better maintenance of fat-free mass.

## METHODS

**Volunteers.** Thirty-six male US Marine Corps infantry officer candidates volunteered to participate in the study. One volunteer was injured during the study, and one refused a post-time-point blood sample, precluding full data collection from these volunteers. Data for the volunteers who completed all aspects of training [ $n = 34$ , 24.5 (SD 0.3) yr, 180.1 (SD 1.1) cm, 83.0 (SD 1.4) kg] were used for statistical comparisons. Potential risks associated with the study were explained, and voluntary written informed consent was obtained from all volunteers before any collection of data. Approval was obtained from the Human Use Review and Scientific Review Committees at the US Army Research Institute of Environmental Medicine and the Human Subjects Research Review Board of its parent organization the US Army Medical Research and Materiel Command (Fort Detrick, MD).

**Experimental design.** A mixed-model, repeated-measures design (diet group  $\times$  time) was used. Volunteers were randomly and blindly

assigned to one of two isocaloric dietary intervention groups. The volunteers were informed that MREs, with or without added protein, would be provided. The supplemental drinks and bars were well matched in terms of color, taste, and appearance; therefore, protein content was effectively blinded. For the group receiving 0.5 g/kg of dietary protein ( $n = 18$ ), 1 of 6 menu selections from the 24-choice menu of MREs that contained marginal amounts of daily dietary protein was provided. In addition, volunteers received a 540-ml chocolate drink that contained 28 g of carbohydrate and 3 g of protein, as well as a 65-g chocolate-flavored bar that provided 28 g of carbohydrate and 3 g of protein. The protein-supplemented group (0.9 g/kg,  $n = 17$ ) received one of six MRE menus with higher amounts of dietary protein, a 540-ml chocolate drink supplemented with protein (28 g of carbohydrate and 18 g of protein), and a protein-fortified chocolate-flavored bar (45 g of carbohydrate and 11 g of protein). Two sources of protein, milk and soy, were used for the protein drink. The powder contained 25% milk protein powder (Alopro 4700, Fonterra) and 15% soy (suproSPI, Archer Daniels Midland). However, each contained nonprotein material: milk powder (70% protein) and soy powder (86% protein). Therefore, 60 g of powder contained 10.5 g of milk protein and 7.7 g of soy protein. Expressed differently, milk protein and soy accounted for 58% and 42% of protein, respectively. The diets of both groups were isocaloric and similar in carbohydrate content (6.3 MJ/day and ~230 g of carbohydrate) but differed in protein content (38 vs. 82 g/day); both diets contained an adequate amount of micronutrients. The rationale for the disparity in protein consumption was that the protein content of the MRE varies considerably and independently of energy content. For example, seven MRE menus contain <34 g of protein (mean 5.3 MJ and 32 g of protein), and seven MRE menus contain >49 g of protein (mean 5.3 MJ and 53 g of protein).

The study was conducted during the US Marine Corps Infantry Officer Candidate School 8-day military field exercise in Quantico, VA. Before the exercise, all subjects were in a controlled environment for 12 wk: they had similar access to a military dining facility for breakfast, lunch, and dinner and underwent similar, structured physical-training programs. The field exercise was scheduled so that activities were conducted in a near-continuous manner, with limited time for sleep (typically between 0200 and 0600). The intensity of similar military activities has previously been reported to be ~35% maximal  $O_2$  consumption (26, 27), with energy expenditures between 12.5 and 20.5 MJ/day (5, 18, 26, 34). Daily ambient temperature ranged from 19.3°C (low) to 29.6°C (high).

**Experimental procedures.** Fasting morning blood samples were collected at the same time before (day 0) and at day 4 (the midpoint) and day 8 (after) of the 8-day exercise. After blood was collected, it was allowed to clot at room temperature. Serum was separated from whole blood by centrifugation at 3,000 rpm and then separated into appropriate aliquots for storage and future analysis. All serum samples were kept frozen at -80°C until analysis was performed. All hormonal variables were performed in duplicate and in a single batch to avoid changes due to interassay variance.

Total IGF-I, free IGF-I, and IGFBP-1, -2, and -3 were measured using a two-site immunoradiometric assay (Diagnostic Systems Laboratories, Webster, TX). ALS was measured using an enzyme-linked immunosorbent assay (Diagnostic Systems Laboratories). The intra-assay variances were 6.1%, 6.1%, 5.0%, and 3.0% for total IGF-I, free IGF-I, IGFBP-1, and IGFBP-3, respectively.

Nonternary IGF-I and nonternary IGFBP-3 (IGF-I + IGFBP-3) were measured using an ALS-immunodepletion assay by methods described elsewhere (14, 18). The immunodepletion assay was performed using a predetermined amount of anti-ALS antibody (Diagnostic Systems Laboratories, Toronto, ON, Canada), which was coupled to a streptavidin-coated magnetic particle and used for immunodepletion of ALS-based complexes. An optimized amount of the antibody-coupled particles (300  $\mu$ l of a 1 mg/ml particle suspension) was added to polystyrene tubes (12  $\times$  75 mm), and the tubes were

placed on the magnetic separation rack for separation of the particles and complete removal of the particles' suspension buffer. Serum samples (20  $\mu$ l) and assay buffer (20  $\mu$ l) were added to each tube, and the samples were allowed to incubate for 2 h with continuous shaking. The tubes were then placed on the magnetic separation rack, and the ALS-depleted samples were assayed for IGF-I and IGFBP-3, as well as ALS, to ensure complete removal of the ALS immunoreactivity.

Total testosterone, free testosterone, androstenedione, dehydroepiandrosterone (DHEA), and DHEA-sulfate (DHEA-S) were measured by competitive radioimmunoassay (Diagnostic Systems Laboratory) using a gamma counter (Cobra, Packard Instruments, Downers Grove, IL). The sensitivity and intra-assay variance were 0.5 ng/dl and 1.6% for total testosterone, 0.18 pg/ml and 7.3% for free testosterone, 3.0 ng/dl and 5.9% for androstenedione, 5.0 ng/dl and 4.6% for DHEA, and 1.7  $\mu$ g/ml and 11.9% for DHEA-S, respectively. Sex hormone-binding globulin (SHBG; Diagnostic Systems Laboratory) was analyzed using a noncompetitive "sandwich" immunoradiometric assay. The sensitivity and intra-assay variance for SHBG was 3.0 nmol/l and 7.1%, respectively. Serum glucose concentrations (Bio-Chem Laboratory Systems, Lakewood, NJ) were measured according to the manufacturer's instructions on an ATAC 8000 instrument (Elan Diagnostics, Smithfield, RI).

Body mass of volunteers wearing shorts only was determined before and after the field exercise with use of a floor scale (model 770, Seca, Hamburg, Germany). Body composition was assessed by whole body dual-energy X-ray absorptiometry (DEXA; Total Body Analysis, version 3.6, Lunar, Madison, WI). Scanning was performed in 1-cm slices from head to toe at a 10-min scanning speed. During intense military training operations, Friedl et al. (4, 6) showed that DEXA-assessed body mass was overestimated by 3–4% and 100 g for fat-free mass in subjects during semistarvation. Therefore, measurements of fat-free mass and fat mass were quantified using DEXA-assessed percent body fat along with floor scale-determined body mass with calculations from the aforementioned investigations.

An ActiGraph (Precision Control Design, Fort Walton Beach, FL) worn on the wrist was used to monitor physical activity, movement, and sleep/rest in a subset of volunteers ( $n = 18$ ) during the exercise. Sleep hours were quantified using the methods of Cole et al. (1). In 12 of the volunteers (6 per diet group), energy expenditure was assessed with the doubly labeled water (DLW) technique using previously described procedures (2, 33). Subjects were dosed using a standard amount of isotope to provide enough isotope for detection of elimination rates for a standard 80-kg man over 8 days with energy expenditure and water turnover rates associated with arduous physical activity in temperatures expected to approximate 30°C (34). Nutrient intake was obtained by collection of empty food wrappers and uneaten food items daily, with the assumption that all items contained in empty food wrappers were consumed by the volunteer. Nutrient intake was quantified from manufacturer-supplied ingredient labels and the MRE nutrient composition database.

**Statistical analysis.** A within-group factor repeated-measures ANOVA was utilized with time as the within-subjects factor and dietary intervention as the between-group factor. Tukey's post hoc test was used to determine statistical differences when ANOVA revealed a significant  $F$  ratio. An alpha level of  $P < 0.05$  was used to denote a significant effect. Kolmogorov-Smirnov tests and visual inspection of normal  $Q-Q$  plots and detrended  $Q-Q$  plots were used to assess the normality of the data. Mauchley's test of sphericity assessed the homogeneity of variance for the data. All statistical analyses were performed using Statistica 6.1 (StatSoft, Tulsa, OK). Values are means (SD).

## RESULTS

Table 1 presents the body composition results. All baseline variables of body composition were similar between diet groups. Body mass declined  $\sim 3.2$  kg (range 0.3–6.1 kg,  $\sim 4\%$

Table 1. Changes in body composition before and after 8 days of sustained military operations

	0.9 g/kg Protein		0.5 g/kg Protein	
	Before	After	Before	After
Body mass, kg	83.0 (9.6)	79.0 (8.9)*	81.6 (5.9)	78.5 (5.7)*
Fat-free mass, kg	69.2 (4.7)	67.5 (8.3)*	69.1 (8.3)	67.8 (7.7)*
Fat mass, kg	13.9 (3.1)	12.5 (3.8)*	12.5 (3.2)	11.0 (2.7)*
Body fat, %	16.6 (3.6)	15.3 (4.2)*	15.1 (3.3)	13.3 (3.0)*
Total body water, liters	45.7 (2.7)	46.5 (2.8)	47.8 (3.4)	48.5 (3.4)

Values are means (SD). Body mass was assessed using a floor scale, and body fat was assessed using dual-energy X-ray absorptiometry. Fat mass was quantified as body mass (kg)  $\times$  body fat (%); fat-free mass was the difference between body mass and fat mass (4, 6). There were no initial differences in body composition variables (by 1-way ANOVA). \*Significant within-group change over the 8 days of the study ( $P < 0.05$ ).

loss of total body mass,  $P = 0.001$ ) over the 8-day study, independent of diet group ( $P = 0.8$ ). The dietary intervention appeared to have no measurable effect on preservation of fat-free mass over the course of the study ( $P = 0.7$ ), inasmuch as both groups lost similar amounts of fat-free mass ( $-1.2$  kg, range  $-5.5$  to  $0.4$  kg,  $P = 0.001$ ). Total body mass losses of 60% and 40% were attributable to fat and fat-free mass tissue losses, respectively. Similarly, both dietary groups lost nearly the same amount of fat mass ( $-1.5$  kg, range  $-3.5$  to  $0.2$  kg,  $P = 0.001$ ). Significant, but modest, Pearson product-moment correlations, suggested that 25% and 21% of the variance in the change in fat-free mass could be explained by the changes in free testosterone and free IGF-I, respectively. Similarly, only 14% of the variance in the change in body mass could be attributed to changes in SHBG concentration.

DLW measurements indicated that daily energy expenditures were similar in both groups ( $P = 0.8$ ), averaging 16.5 (SD 2.0) MJ/day (range 14.4–19.9 MJ/day) and 15.9 (SD 2.1) MJ/day (range 13.9–17.4 MJ/day) for 0.5 and 0.9 g/kg, respectively. Over the first 3 days of the experimental period, volunteers slept an average of 4.3 (SD 0.8) h/day. Because of malfunction of the ActiGraphs, insufficient data were available to quantify sleep on days 4 and 8. Daily energy intake averaged 6.5 (SD 0.6) and 6.4 (SD 0.7) MJ/day for 0.5 and 0.9 g/kg, respectively, and total energy intake over the 8-day study period was similar ( $P = 0.8$ ) between groups. There were no statistical differences between dietary groups ( $P = 0.5$ ) for energy deficit, which averaged 9.7 MJ/day. Daily carbohydrate intake averaged 211 (SD 49) and 207 (SD 30) g for 0.5 and 0.9 g/kg, respectively. There was a significant main effect of group for dietary fat in the groups receiving less protein:  $37.7 \pm 2.1$  and  $44.7 \pm 2.0$  g for 0.5 and 0.9 g/kg, respectively ( $P = 0.029$ ). There was no significant interaction over the course of the study between groups for fat ( $P = 0.9$ ), whereas daily dietary protein intake differed significantly: 37 (SD 5) and 77 (SD 11) g for 0.5 and 0.9 g/kg, respectively ( $P = 0.001$ ). Daily protein intake normalized for body mass averaged 0.5 (SD 0.1) and 0.9 (SD 0.2) g/kg, respectively.

The IGF-I system responses are shown in Fig. 1. Total IGF-I declined over the 8-day study period, falling  $\sim 50\%$  in both groups by day 4 ( $P = 0.001$ ), with an interaction ( $P = 0.01$ ) between the diet groups' response from day 4 to day 8. Subsequent post hoc analysis revealed that total IGF-I declined from day 4 to day 8 in the 0.5 g/kg group but did not change

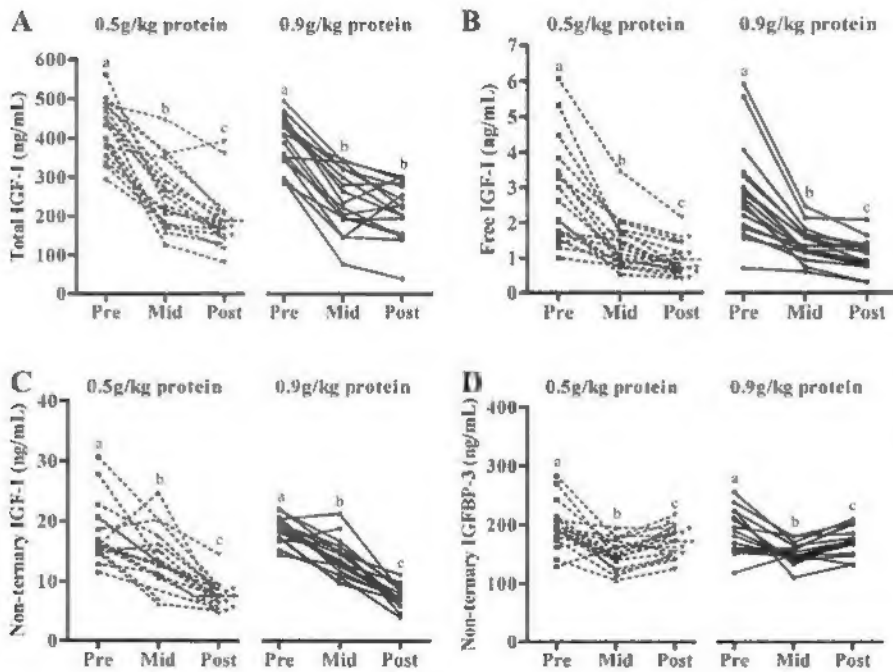


Fig. 1. Total insulin-like growth factor (IGF; A), free IGF-I (B), nontertiary IGF-I (C), and non-tertiary IGF-binding protein-3 (IGFBP-3; D) responses to dietary protein intake of 0.5 g/kg body wt (dashed lines) and 0.9 g/kg body wt (solid lines) before (Pre), at the midpoint (Mid), and after (Post) the 8-day field exercise. Different letters (a, b, c) denote within-subjects statistical difference ( $P < 0.05$ ); similar letters denote no statistical differences. There were no initial differences in IGF-I system analytes between groups (1-way ANOVA). Values are means (SD).

in the 0.9 g/kg group. Free IGF-I ( $P = 0.001$ ; Fig. 1B) and nontertiary IGF-I ( $P = 0.001$ ; Fig. 1C) were significantly lower than baseline concentrations on day 4 and were further reduced on day 8 but were not different between groups. The decrease in free IGF-I and nontertiary IGF-I was 100% and 140%, respectively, by the completion of the 8-day study. Nontertiary IGFBP-3 (IGF-I + IGFBP-3) concentrations declined 35% by day 4 ( $P = 0.01$ ; Fig. 1D), with no significant differences between diet groups.

Responses of IGFBPs and ALS are presented in Fig. 2. IGFBP-1 increased threefold by day 4 and remained elevated to day 8 ( $P = 0.001$ ; Fig. 2A), with no differences between diet groups. There was a significant negative correlation after ( $r = -0.50$ ), but not before ( $r = -0.07$ ), training between IGFBP-1 and glucose. IGFBP-2 exhibited a biphasic response in both groups, with a twofold decrease by day 4 ( $P = 0.001$ ; Fig. 2B), but was similar to original values by day 8. IGFBP-3 and ALS decreased progressively over the 8-day study period ( $P =$

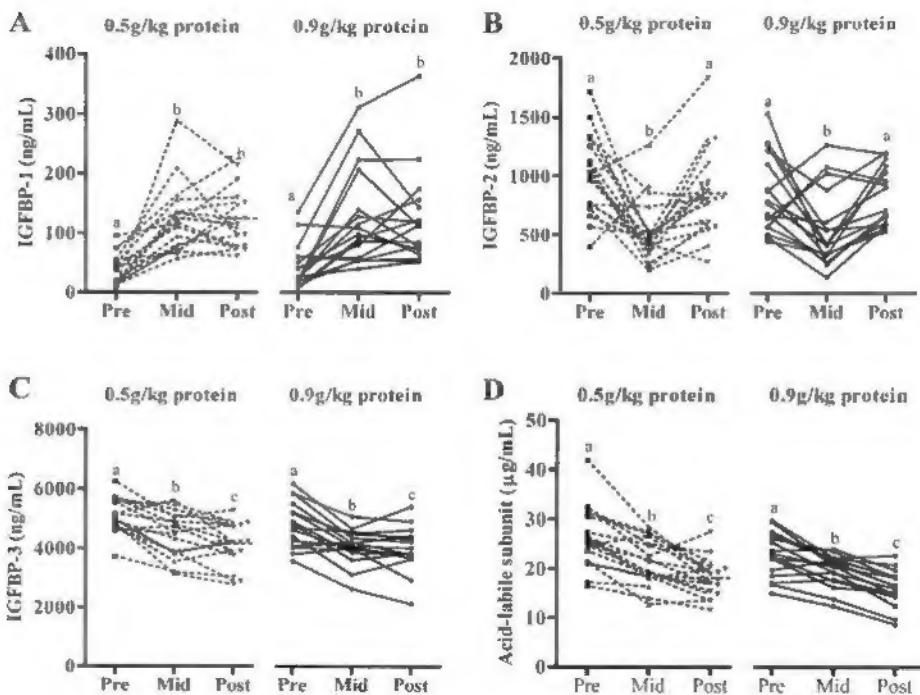


Fig. 2. Responses of IGFBP-1 (A), IGFBP-2 (B), IGFBP-3 (C), and acid-labile subunit (D) to the 8-day energy deficit during dietary protein intake of 0.5 g/kg body wt (dashed lines) or 0.9 g/kg body wt (solid lines). Different letters denote within-subjects statistical difference ( $P < 0.05$ ); similar letters denote no statistical differences. There were no initial differences in IGF-I system analytes (1-way ANOVA). Values are means (SD).

0.001; Fig. 2, *C* and *D*), with no difference between diet groups.

Testicular and adrenal androgen responses are shown in Fig. 3. Total and free testosterone were 49% and 60% lower, respectively, on *day 4* and remained depressed through *day 8* ( $P = 0.001$ ; Fig. 3, *A* and *B*). Total testosterone ( $P = 0.3$ ) and free testosterone ( $P = 0.7$ ) did not differ significantly between groups. Relative to *day 0* concentrations, SHBG was increased by *day 8*. However, the magnitude of the increase depended on diet: SHBG was higher in the 0.5 g/kg than in the 0.9 g/kg group ( $P = 0.001$ ). DHEA and androstenedione declined 100% and 60%, respectively, by *day 4* and remained lower than baseline at *day 8* ( $P = 0.001$ ; Fig. 3, *D* and *E*), independent of diet group ( $P = 0.4$ ). DHEA-S was elevated 27% by *day 4*; however, it returned to baseline by the end of the study period ( $P = 0.003$ ; Fig. 3*F*). No significant changes were evident between diet groups for DHEA-S ( $P = 0.9$ ).

## DISCUSSION

The purpose of the present study was to assess whether changing the level of dietary protein from 0.5 to 0.9 g/kg body wt would attenuate the decline in anabolic hormones and, subsequently, mitigate losses of fat-free mass during a negative energy balance produced by high energy expenditure coupled with low energy intake. We identified a military population that performed significant amounts of physical labor without sufficient rest or the ability to eat enough to achieve energy balance. Dietary protein content was manipulated without affecting dietary carbohydrate levels, and energy expenditure was precisely monitored by the DLW technique in a representative sample of study participants (33).

The key findings of the present study are as follows: 1) consumption of a low-energy adequate-protein diet produced a subtle attenuation in the decline of total IGF-I and blunted the increase in SHBG; 2) the IGF-I and androgenic systems were altered, independent of dietary manipulation, within 72 h; and 3) the difference in dietary protein intake had no measurable effect in the ability to attenuate the loss in fat-free mass. From these collective findings, we conclude that, under the severe energy deficit (9.6 MJ/day) conditions, although dietary protein intake of 0.5 or 0.9 g/kg body mass subtly attenuated IGF-I and decreased SHBG, it failed to exert a more global influence on anabolic growth factors and did not result in preservation of fat-free mass.

A priori, we hypothesized that a dietary protein intake of 0.5 g/kg would exacerbate the decline of circulating total and free IGF-I. However, the results suggest that manipulation of daily dietary protein intake between 0.5 and 0.9 g/kg body mass had minimal impact on the IGF-I system response to an 8-day energy deficit. Although the higher-protein diet demonstrated a subtle attenuation in total IGF-I, the response of bioavailable IGF-I, specifically free and nontertiary IGF-I, was not affected by the level of protein intake. Therefore, the biological significance of the attenuation of total IGF-I is not clear. Perhaps the energy deficit was too severe for preservation of IGF-I by protein supplementation at any level.

The negative energy balance noted in the present investigation may have exceeded a threshold whereby protein intake would effectively attenuate the decline of bioavailable IGF-I. Total energy intake between 0.06 and 0.1 MJ/day appears to sustain circulating IGF-I, independent of protein intake during refeeding after a 5-day fast (11) and limited sleep (23). Furthermore, IGF-I concentrations are better maintained (i.e.,

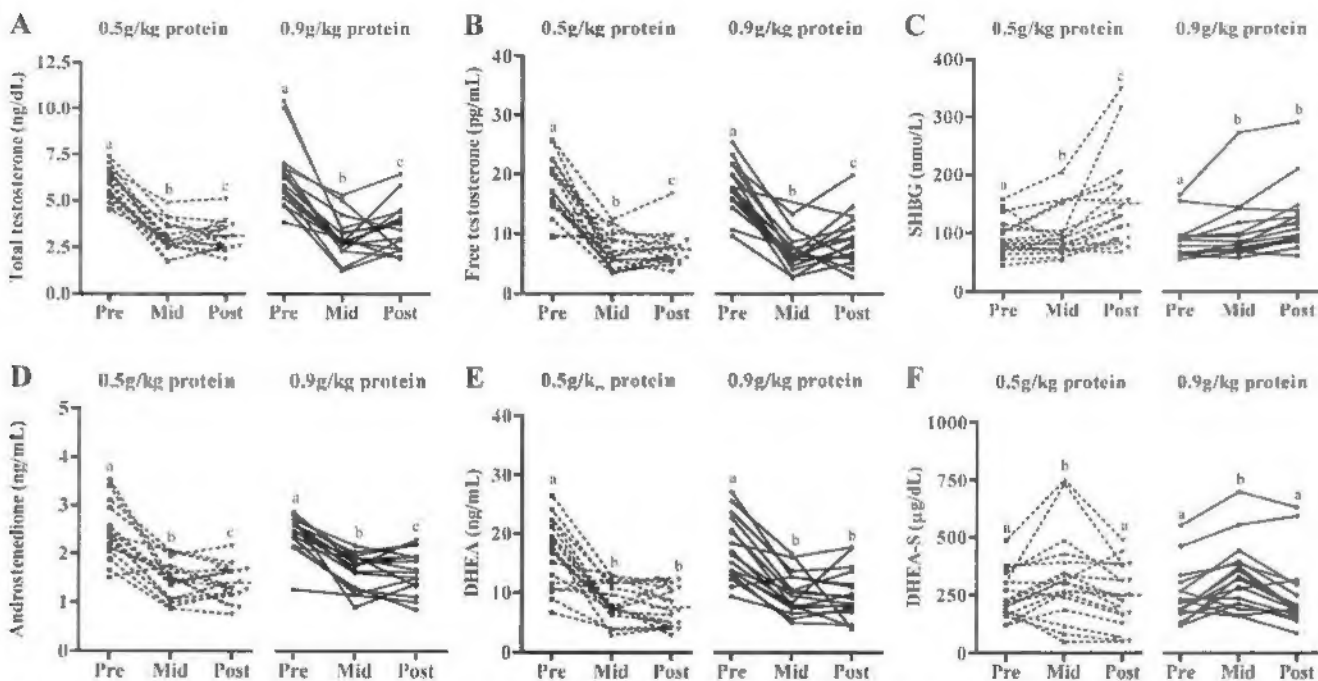


Fig. 3. Responses of testicular and adrenal androgens to 0.5 g/kg dietary protein (dashed lines) and adequate dietary protein (solid lines) groups before, at the midpoint, and on completion of the 8-day field exercise for total testosterone (*A*), free testosterone (*B*), sex-hormone binding globulin (*C*), androstenedione (*D*), dehydroepiandrosterone (DHEA; *E*), dehydroepiandrosterone-sulfate (DHEA-S; *F*). Different letters denote within-subjects statistical difference ( $P < 0.05$ ); similar letters denote no statistical differences. There were no initial differences in testicular and adrenal androgen analytes (1-way ANOVA). Values are means (SD).

normal physiological concentration) by a diet that is adequate in total energy than by a diet that is low in energy and protein (11, 29). Recently, Rarick et al. (32) suggested that, during energy balance, with an exercise-induced increase in normal activity, IGF-I concentrations can still be suppressed by 30%, possibly by "increased energy flux." Our results are also consistent with those of Nindl et al. (18), who reported reductions in total and free IGF-I within 3 days of energy restriction (6.5 MJ/day and 0.8 g/kg of dietary protein). Similar decreases of 43% in IGF-I have been demonstrated after a 5-day French Commando course in which energy intake was limited to 9.2 MJ/day and energy expenditure was 20.9 MJ/day (7). Similar to the decline in IGF-I, with adequate nourishment, IGF-I is rapidly restored (5, 10, 11, 16, 21).

IGF-I binding proteins were also significantly altered during our experimental paradigm. IGFBP-1 increased 85% over the course of the present study. This small-molecular-weight (23.5-kDa) binding protein responds rapidly to fasting or feeding and is thought to be important in glucose regulation and to inhibit the actions of IGF-I on target tissues (13). The observation that the changes in IGFBP-1 were correlated with changes in blood glucose (after, but not at the beginning of, the study) is consistent with our interpretation that the IGFBP-1 response is a physiological adaptation to preserve glycemia. The singular binding proteins of the ternary complex, IGFBP-3 and ALS, were significantly decreased within 72 h of the field exercise and directionally tracked the changes in total and free IGF-I. Nonternary IGF-I and nonternary IGFBP-3 were decreased throughout the study, but the relative distribution of IGF-I between ternary, nonternary, and free IGF-I remained unchanged. Our results are consistent with those from a similar model (18) but are different from findings reported by Thissen et al. (35) and Norrelund et al. (22), who reported a shift from high- to low-molecular-weight complexes during long-term fasting and/or protein deprivation. The disparate outcomes between studies might be explained by periodic feeding vs. total fasting or methodological differences in measurement of various molecular weight complexes between studies.

Dietary protein content did modify the SHBG response to arduous work and energy restriction. SHBG concentration only increased 23% above baseline levels in the higher-protein group, whereas SHBG increased 66% in the 0.5 g/kg group, resulting in 42% lower SHBG in the 0.9 g/kg group than in the group receiving less protein during 8 days of energy deficit. Increases in SHBG concentration have been observed in other studies that included energy restriction and high levels of physical activity (5, 15). Moreover, inverse relationships between dietary protein intake and SHBG have been reported in animal and human models (15). Inasmuch as SHBG controls the majority of the circulating pool of testosterone, elevated SHBG concentrations (9) are known to inhibit bioavailable testosterone. The blunted SHBG response in the group given more protein would have been expected to translate into attenuation of the decline in bioavailable testosterone. However, free testosterone levels were similar between diet groups, suggesting that the lower SHBG had a limited effect on circulating free testosterone accompanying arduous military training.

When DHEA-S increases, it increases only for a few days (26) and returns to baseline with increasing duration of stressors. Mechanisms underlying this transient increase in hor-

mone are postulated to be an upregulation of peripheral tissue sulfokinase activity, a decrease in the desulfation mechanism of the liver, or differential secretion rates of DHEA or DHEA-S. Under similar conditions, Opstad and Aakvaag (26, 28) suggested that the rapid increase in DHEA and DHEA-S concentration may serve to counteract the fall in testosterone. The increase in adrenal androgens did not accommodate the concomitant decrease in testicular androgens, inasmuch as the stress was prolonged and severe.

The time course and magnitude of decline of total (~49%) and free testosterone (~60%) are similar to previous studies (5, 8, 17–19, 26–28). Our observation that manipulation of dietary protein had no impact on the testosterone response suggests that dietary protein intake of 0.5 or 0.9 g/kg body wt during a short-term energy deficit (~10 MJ/day) results in similar outcomes. However, it must be recognized that the dietary intervention involved only modest differences in protein consumption between groups. It has been postulated that the decline of testosterone contributes to preservation of normoglycemia by enhancing alanine availability for gluconeogenesis (24, 25). The maintenance of normal glucose concentrations during these times is important given the lack of time to eat and the paucity of food.

In the present study, the energy deficit was within the range (~5.0–17.0 MJ/day) encountered in previously conducted studies under similar conditions (34). Contrary to our hypothesis that insufficient protein contributes to loss of lean mass during energy imbalance, both dietary groups lost similar amounts of fat-free mass. Better maintenance of fat-free mass and nitrogen retention may occur during energy restriction when dietary protein intake is 1.5 g/kg body wt (3, 31). Thus one explanation for our lack of differences between diet groups could be that supplementation of the diet to achieve the US Recommended Dietary Allowance of dietary protein was insufficient during such severe periods of energy restriction and high energy expenditure to mitigate loss of fat-free mass loss. In conclusion, dietary protein up to levels equivalent to the Recommended Dietary Allowance (0.9 g/kg body wt) during a period of substantial physical activity and energy deficit had limited effects on the IGF-I and androgenic systems, as well as retention of fat-free mass. These observations suggest that modest manipulation of dietary protein (0.5 vs. 0.9 g/kg) during arduous physical labor coupled with low energy intake has minimal physiological impact over 8 days of arduous physical activity.

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